**Table SM1: Summary of *in vitro* studies involving exposure to iron oxide particles. Abbreviations: ALP – alkaline phosphatase; AM – alveolar macrophages; ATP – adenosine triphosphate; BAL – bronchoalveolar lavage; CB – carbon black; DLS – dynamic light scattering; EC – endothelial cell; ECM – extracellular matrix; ER – endoplasmic reticulum; GSH – glutathione; ICAM – intercellular adhesion molecule; IL – interleukin; LDH – lactate dehydrogenase; MDA – malondialdehyde; ND – not determined; NPs – nanoparticles; PGE2 – prostaglandin-E2; ROS – reactive oxygen species; SEM – scanning electron microscopy; SPION – superparamagnetic iron oxide particles; SSA – specific surface area; TEM – transmission electron microscope; TNF – tumour necrosis factor; VSOP - very small superparamagnetic iron oxide particle; Zeta – Zeta potential.**

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| **Particle Type and Reference** | **Particle Properties, Size, and Exposure Conditions** | **Particle Source** | **Cell Type** | **Toxicity Results** |
| Nano and bulk (micro) Fe3O4 particles(Könczöl et al. 2011) | Fe3O4 nano: 20-60 nm (SEM).Fe3O4 bulk: 0.2-10 µm (SEM).Exposure concentration: 0 – 200 µg/ml for 6-24 hour exposure. | Purchased from chemical supplier (Alfa Aesar, Germany & Sigma-Aldrich USA). | Human lung epithelial cells (A549). | * TEM showed smaller component of bulk magnetite (<1 µm) incorporated into membrane bound vesicles as small aggregates.
* NPs incorporated into membrane bound vesicles in large aggregates (up to hundreds of particles), smaller aggregates were found in the cytoplasm, only 1 particle was in the nucleus.
* Uptake appears to be via phagocytosis or micropinocytosis.
* 80% cell viability for 200 µg/ml bulk (loss of 20%).
* ROS formation significantly increased following 24 hr exposure to bulk (1.4 fold) and nano (1.3 fold) at 100 µg/ml.
* Smaller particles (nano and bulk <1 µm) produced genotoxic effects as confirmed by DNA migration via Comet assay. These effects were partially inhibited by ROS scavengers.
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| Bulk and nano Fe3O4 particles(Könczöl et al. 2013) | Bulk Fe3O4: 0.2 – 10 µm mean diameter; SSA 0.99 m2/g.Nano Fe3O4: <50 nm mean diameter; SSA 40 m2/g.Exposure concentrations: 10 and 100 µg/cm2. | Bulk magnetite purchased from chemical supplier (Alfa Aesar, Germany); nanoparticle magnetite purchased from chemical supplier (Sigma Aldrich, Germany). | Human lung epithelial cells (A549) and Human lymph node cells (H1299 which do not express p53). | * Significant increase in ROS formation for bulk magnetite at 100 µg/cm2 and NPs at both concentrations tested (>9 fold increase for 100 µg/cm2 NP magnetite).
* 1.59 fold increase (statistically significant) in catalase activity following A549 cell exposure to 100 µg/cm2 NP magnetite.
* Reduction in GSH for all NP concentrations tested but it did not reach significance.
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| Nano Fe3O4 particles(Liu et al. 2012) | Fe3O4: 25 nm (TEM); Zeta -12 mV (ECM); Zeta -15 mV (RPMI medium); SSA ~38.7 m2/g.Exposure concentration: 0 – 400 µg/ml for 24 hour exposure. | Purchased from chemical supplier (Sigma-Aldrich, USA). | Human umbilical vein endothelial cells (HUVEC) & human monocyte cell line (THP-1). | * Exposure to 200 µg/ml or greater caused significant cytotoxic effects in HUVECs but there was no significant loss of viability in the THP-1 cell line at any of the concentrations tested.
* TEM analysis showed protrusion of the cell membrane in THP-1 cells but not HUVECs suggesting that incorporation of particles into monocytes is via phagocytosis but in ECs it might be via other endocytic pathways.
* High concentration of particles in lysosomes, but no particles were found in the nucleus or mitochondria.
* Lysosomal morphology changes were seen in HUVECs but not THP-1 cells, which might be a possible pathway for cytotoxicity via lysosomal injury.
* Exposure to Fe3O4 particles showed no significant effect on endothelial cell adhesion molecule expression or cytokine release.
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| Nano Fe3O4 particles(Szalay et al. 2012) | Fe3O4 nano: <50 nm (supplier quoted); SSA 60 m2/g.Exposure concentration: 0 – 10,000 µg/ml for 4 or 24 hour exposure. | Purchased from chemical supplier (Sigma-Aldrich, USA). | Vero cell line (C1008). | * Particles displayed cytotoxicity at concentrations ≥2500 µg/ml (these concentrations are 5-fold higher than those in most other studies).
* They showed no mutagenicity.
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| Nano Fe3O4 particles (coated and uncoated tested, results presented for uncoated)(Baber et al. 2011) | Fe3O4: 25 nm diameter.Exposure concentration: 5.0 g/cm3 exposure of cells at air-liquid interface to airborne iron oxide particles.Exposure concentrations: 4, 20, 100 µg/ml. | Purchased from chemical supplier (uncoated – Sigma Aldrich; coated – Degussa AG). | Human bronchial epithelial cells (BEAS-2B) | * Solubility of both Fe2+ and Fe3+ increased with increasing acid strength (eg. no acid: Fe2+ 0.039 ng, Fe3+ 0.005 ng and 20% sulfuric acid: Fe2+ 1.391 ng; Fe3+ 0.598 ng).
* There was a significant decrease in cell viability for exposure to Fe3O4 particles in the presence of acid but not with no acid with 60% cell viability seen for 250 µg/ml particles in the presence of 20% sulfuric acid.
* Uncoated particles in the presence of 20% sulfuric showed a significant decrease in cell viability from a particle concentration of 50 µg/ml.
* IL-6, IL-8, TNFα and heme oxygenase-1 all showed a significant increase over control (40-90% increase) when exposed to iron oxide particles with acid but not without acid.
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| Nano Fe3O4 particles(Wu et al. 2013) | Fe3O4: diameter 30 nm (TEM); Zeta potential -9.1 mV.Exposure concentrations: 25 – 200 µg/ml for 24 hour exposure. | Purchased from chemical supplier (Sigma Aldrich) | Rat adrenal medulla cells (PC12).  | * Significant cytotoxicity and LDH leakage in PC12 cells following 24 hour exposure at 100 or 200 µg/ml concentrations.
* Significant increase in ROS from 25 µg/ml.
* GSH and superoxide dismutase showed a dose dependent decrease, significant from 50 µg/ml. As well as an increase in MDA concentration.
* The cell death rate increased from 1.84% to 16.25% following 200 µg/ml particle exposure.
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| Nano Fe3O4 particles(Dwivedi et al. 2014) | Fe3O4: 22 nm diameter (TEM).Exposure concentrations: 10 – 50 µg/ml for 24 hours.  | Synthesized: by precipitation of FeSO4 and FeCl3 with NH4OH. | Human lung epithelial cells (A549) | * Significant decrease in cell viability (via MTT assay) from 10 µg/ml down to a maximum of 56% cell viability for 50 µg/ml.
* Significant decrease in cell viability (via NRU assay) from 10 µg/ml down at a maximum of 65% at 50 µg/ml.
* Concentration dependent increase in lipid peroxidation up to a maximum of 91% at 50 µg/ml.
* Significant decrease in GSH (54% decrease for 50 µg/ml).
* Statistically significant (p<0.001) increase in ROS generation up to 82% increase for 50 µg/ml exposure.
* Statistically significant concentration dependent decrease in mitochondrial membrane potential up to a 62% decrease for 50 µg/ml.
* An alteration in cellular morphology in cells exposed to Fe3O4 particles.
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| Nano Fe3O4 particles (PAA-coated and uncoated)(Couto et al. 2015) | Non-coated Fe3O4 NPs: 9.9 nm average size; Zeta potential -1.91 mV.PAA-coated Fe3O4: 10.0 nm average size; Zeta potential -3.01 mV. Both particles showed agglomeration in RPMI medium 1640 medium.  | Synthesized: from Fe(II) and Fe(III) chloride salts, precipitated using NH4OH. Polyacrylic acid (PAA) added for coated particles. | Cell culture of T-cells from human blood donors. | * Uncoated Fe3O4 NPs showed a small increase in genotoxicity in the presence of bleomycin (BLM), however, it did not reach significance for any concentration.
* Interestingly, the highest number of strand breaks per cell was for the lowest uncoated particle concentration (4 µg/ml).
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| Fe3O4 particles in the presence of acid and/or secondary organic aerosol(Ghio et al. 2009) | Fe3O4: diameter 80-100 nm.Exposure concentrations: Cells were exposed to nebulized particles. Dose 20 – 40 µg either Fe3O4 only; Fe3O4 plus sulfuric acid; Fe3O4 plus secondary organic aerosol (SOA); Fe3O4 plus acid plus SOA.  | Purchased from chemical supplier (Aldrich, USA). | Human bronchial epithelial cells (BEAS-2B). | * Exposure to Fe3O4 particles, Fe3O4 + acid, Fe3O4 + SOA all significantly increased the intracellular iron concentration.
* The highest intracellular iron concentration seen for iron oxide + acid + SOA.
* Cellular ferritin didn't increase with Fe3O4 particles alone but did significantly increase for Fe3O4 in the presence of either acid or SOA or both.
* Acetaldehyde increased 6 hours after exposure for Fe3O4 in the presence of acid or SOA or both but not Fe3O4 particles alone.
* IL-8 levels in the cell supernatant were significantly increased for Fe3O4 particles in the presence of acid or SOA or both.
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| Nano and micro Fe2O3 and Fe3O4 particles(Guichard et al. 2012) | Fe3O4 nano: 27 nm (TEM); SSA 40 m2/g.Fe3O4 micro: 156 nm (TEM); SSA 7 m2/g.Fe2O3 nano: 35 nm (TEM); SSA 39 m2/g.Fe2O3 micro: 147 nm (TEM); SSA 6 m2/g.Exposure concentration: 0 – 200 µg/cm2 for 24 or 72 hour exposure. | Purchased from chemical supplier (Sigma-Aldrich, France). | Syrian hamster embryo cells (SHE). | * All particles showed uptake into the SHE cells.
* 200 µg/cm2 iron oxide particles produced no significant ROS on their own in an acellular assay. In the presence of H2O2 all particles showed a significant increase in ROS production, with the nano particles producing more ROS and all iron oxides producing more ROS than TiO2 particles under equivalent conditions.
* EC50 (72 hr exposure) for Fe2O3 nano was 65.1 µg/ml.
* None of the particles showed DNA tail damage or micronucleus formation.
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| Micro Fe2O3 and Fe3O4 particles(Breznan et al. 2013) | Fe2O3: <5 µm (supplier quoted).Fe3O4: <5 µm (supplier quoted).Exposure concentration: 0 – 667 µg/ml for 2 hour exposure. | Purchased from chemical supplier (Sigma-Aldrich, USA). | Alveolar macrophages (BAL extraction from euthanized Fischer rats). | * Fe2O3 and Fe3O4 produced a significant decrease in respiratory burst. Subsequent exposure to a stimulant resulted in a decrease in stimulant-induced burst.
* This response could potentially result in a decrease in innate immunity within the lung.
* The metal oxide particles with the highest potency were those with insoluble components capable of generating ROS.
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| Submicro Fe2O3 and Fe3O4 particles co-administered with cristobalite(Williams and Zosky 2019) | Fe2O3 and Fe3O4 particle size ranged from 0.2 to0.8 μm aerodynamic diameter.Exposure concentrations: 0.38 - 57 µg/ml. Study used very low concentrations of particles.The particles were administered as combinations of cristobalite with either Fe2O3 or Fe3O4 for 24 hours exposure in BEAS-2B cells. | Purchased from chemical supplier (Sigma-Aldrich) | Human lung epithelial cells (A549) and human bronchial epithelial cells (BEAS-2B). | * There was no observed cytotoxicity, LDH increase, IL-6 or IL-8 increased for any concentration tested for either particle (note that Cristobalite and quartz also failed to increase LDH).
* The combinations of iron oxide and cristobalite in BEAS-2B cells did not induce a significant change in LDH levels over the cristobalite-induced response.
* 38 µg/ml cristobalite with 1.9 µg/ml or 19 µg/ml hematite both caused an increased in IL-6 and IL-8 compared to cristobalite alone but this increase was not significant.
* Magnetite in combination with cristobalite also caused an increase but this was smaller than that seen for hematite.
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| Nano and micro Fe2O3 and Fe3O4 particles(Karlsson et al. 2009) | Fe2O3 nano: 30-60 nm (TEM); SSA 40 m2/g.Fe2O3 micro: 0.150-1 µm (TEM); SSA 5.4 m2/g.Fe3O4 nano: 20-40 nm (TEM); SSA 42 m2/g.Fe3O4 micro: 0.1-0.5 µm (TEM); SSA 6.8 m2/g.Exposure concentration: 40 µg/ml & 80 µg/ml for 18 hour exposure. | Purchased from chemical supplier (Sigma-Aldrich, USA). | Human lung epithelial cells (A549). | * In solution the NPs formed agglomerates which put their total size in the micro range.
* Iron oxide particles showed very little toxicity.
* No evidence that nano iron oxides particles were more toxic than micro, this could be related to the agglomeration.
* Iron oxide showed slight mitochondrial damage (~10-15% depolarized cells for all particle types which was significantly higher than the control but significantly less toxic than CuO particles).
* Nano Fe3O4 showed significant oxidative DNA damage compared to the control (~15%).
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| Nano Fe2O3 and Fe3O4 particles(Sun et al. 2011) | Fe2O3 nano: 42.5 nm (TEM); Zeta -8.2 mV; SSA 82.8 m2/g.Fe3O4 nano: 46.8 nm (TEM); Zeta 1.7 mV; SSA 147.4 m2/g.Exposure concentration: 0 – 100 µg/ml for 12 or 24 hour exposure. | Purchased from chemical supplier (Sigma-Aldrich, USA). | Human cardiac microvascular endothelial cells (HCMEC). | * No cytotoxicity was seen for either iron oxide particle (determined by LDH leakage).
* There was no significant increase in ROS.
* No increase in endothelial cell permeability.
* No significant increase in the mRNA expression of a range of inflammatory markers.
* The iron oxides were the least toxic of the metal oxide nanomaterials in this study (ZnO, CuO, MgO), the authors correlated this to their larger specific surface area.
* There was no correlation between Zeta potential and cytotoxicity in this study.
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| Nano Fe2O3 and Fe3O4 particles(Zhu et al. 2011) | Fe2O3 nano: 22 nm (supplier quoted).Fe3O4 nano: 43 nm (supplier quoted). Exposure concentration: 0 – 200 µg/ml for 6, 12 or 24 hour exposure. (FeCl3 4, 40, 200 µg/ml). | Purchased from chemical supplier (Nanjing Haitai Nanomaterial Co. Ltd. & Sigma-Aldrich, USA). | Human aortic endothelial cells (HAEC) and Monocytic cells (U937). | * Significant dose dependent cytotoxicity with a decrease in cell viability in both cell lines.
* FeCl3 showed the greatest decrease in cell viability.
* Effects varied greatly by concentration and time, with the Fe2O3 at 100 µg/ml 24 hours after exposure in HAECs showing only ~30% viability.
* Significant increase in ROS for all particles at concentrations > 20 µg/ml lasted at least 6 hours following a 10 minute exposure.
* Both NPs were found localized in HAECs (TEM) with visible mitochondrial swelling and disappearance of mitochondria.
* Increase in the attachment of U937 cells to HAECs after 4 hours of co-incubation with 20 µg/ml iron oxide NPs.
* Lower concentrations had significantly better solubility. Following 6 hours of 0.2 µg/ml exposure 23% of Fe2O3 was dissolved and 56% of Fe3O4 was dissolved, which increased to >80% at 24 hours. Whereas, only 1.48% of the 20 µg/ml exposure to Fe2O3 and 1.3% of Fe3O4 at 24 hours was dissolved. This may explain the lack of correlation between concentration, time and effect.
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| Nano Fe2O3 (maghemite) and Fe3O4 particles(Park et al. 2014a) | Fe3O4 nano: 41.5 nm spherical (hydrodynamic diameter in vehicle TEM); Zeta -50.52 mV (vehicle) and -7.38 mV (culture).γ-Fe2O3 nano: 101 nm needle-like shape (TEM), Zeta -28.97 mV (vehicle) and -9.92 mV (culture).Exposure concentration: 6.25 – 50 µg/ml for 24 hour exposure. | Synthesis: iron oleate, oleic acid and 1-octadecene at 320 °C then bound to phospholipid. | Murine alveolar macrophage cell-line (MH-S). | * MH-S cells showed uptake of both NPs over time.
* Fe2O3 NPs easily penetrated the cell membrane and formed large vacuoles in the cytosol, they then induced the unification of the vacuole leading the disappearance of cytosolic organelles though the cellular membrane remained intact.
* Fe3O4 NPs internalization of organelles into the phagosome was more pronounced.
* Both NPs showed a concentration dependent decrease in cell viability. Fe3O4 NPs showed a significant decrease from 6.25 µg/ml and Fe2O3 cells showed a significant decrease from12.5 µg/ml.
* When the cell line was exposed to 50 µg/ml NPs cell viability decreased to 11.0% for Fe3O4 and to 60.0% for Fe2O3.
* Fe2O3 particles increased the number of cells in the G1 phase whereas Fe3O4 NPs increased the number of cells in the G2/M phase.
* Both NPs caused a decrease in ATP production, and damage to the ER though this was higher for Fe3O4 particles.
* Both particles showed a significant increase in ROS with Fe3O4 showing a slightly higher response (50 µg/ml exposure: 241% for Fe3O4 and 172% for Fe2O3).
* Both NPs also caused an increase in cytokine activity.
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| Micro Fe2O3 particles (2 different sizes) (Beck-Speier et al. 2009) | Fe2O3 micro: 0.5 µm (TEM); SSA 17 m2/g.Fe2O3 micro: 1.5 µm (TEM); SSA 7 m2/g.Exposure concentration: 0 – 10 µg/ml for 254 hour exposure for IL-6 & PGE2 study; 1:1 particle:AM for 10 day exposure solubility study. | Flame synthesis: Solution of Fe2(NO3)3 dropwise into a tube furnace at 800 °C. | Alveolar macrophages, BAL extracted from Wistar Kyoto rats | * Both particles showed moderate solubility in alveolar macrophages despite being insoluble in extracellular media.
* 1.5 µm particles induced both IL-6 (1.8 fold) and PGE2 (1.9 fold) production. Suggests soluble Fe arising from dissolved iron particles within AM modulates IL-6 production via PGE2.
* 0.5 µm particles only induced PGE2 synthesis (2.5 fold).
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| Nano and micro Fe2O3(hematite) particles(Bhattacharya et al. 2012) | α-Fe2O3 nano: 25-50 nm (SEM); Zeta -30.4 mV (milli q), -9.35 mV (DKSFM (Defined keratinocyte serum–free medium) + 0.1% protein).α-Fe2O3 micro: ≤500 nm (SEM); Zeta -23 mV (milli q), -11.1 mV (DKSFM + 0.1% protein).Exposure concentration: 10 – 250 µg/ml for 48 hour exposure. | Purchased from chemical supplier (Sigma-Aldrich). | Human lung fibroblasts (IMR-90) and human bronchial epithelial cells (BEAS-2B, SV40 virus transformed). | * Both particles were found in the cell in close proximity to the mitochondria but none were found inside the mitochondria or nucleus.
* Higher agglomeration with NPs c.f. micro particles likely due to an increase in van der Waals forces and altered agglomeration in different culture mediums and different pH.
* Leachable Fe3+ content was higher in NPs, possibly due to higher surface area.
* Both NP and micro particles were shown to generate acellular ROS.
* Both NP and micro particles induced significant cytotoxicity and genotoxicity in vitro (in both cell lines). 250 µg/ml NPs induced ~30% cell death in BEAS-2B cells.
* NPs had an EC50 of 148 µg/ml in IMR-90 cells but only reached a maximum of 40% reduction in cell viability at 250 µg/ml in BEAS-2B cells.
* Micro particles were less toxic than NPs but still showed a significant decrease in cell viability at all concentrations in BEAS-2B cells.
* Changes to chemical environment modified toxicity through alterations in agglomeration.
* Toxicity was mediated through ROS which was modulated by free surface area.
* Concentrations of >50 µg/ml were required to induce toxicity, suggest this is unlikely to be encountered.
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| Micro, nano and rod-shaped Fe2O3 particles(Lee et al. 2014) | Micro Fe3O4: <1000 nm; SSA 5.4 m2/g; size distribution 423 nm (in water); Zeta potential 33.8 mV (in water).Nano Fe3O4: 36 nm, SSA 32 m2/g; size distribution 284 nm (in water); Zeta potential 42.1 mV (in water).Rod-shaped Fe3O4: >500 nm length, 50-100 width, SSA 109 m2/g; size distribution 614 nm (in water); Zeta potential 42.0 mV (in water).Exposure concentrations: up to 100 µg/ml for 24 hours. | Micro and nano sized Fe2O3 were purchased from chemical supplier (Sigma-Aldrich); Rod-shaped Fe2O3 were purchased from chemical supplier (Nano Technology). | Rat macrophage cell line (RAW 264.7) | * TEM determined that particles were located in the perinuclear area and around the cytoplasm, micro and nano were found mostly in vacuoles, however rod shaped particles were found throughout the cytoplasm.
* WST-1 viability assay showed decreasing cell viability with increasing concentration with nano and rod-shaped > micro. However, the LDH assay showed that the rod-shaped particles were significantly more cytotoxic that the other particles (almost double the cytotoxicity of nano and micro at 200 µg/ml).
* At 200 µg/ml all particles showed an increase in TNF-α, rod-shaped particles showed largest increase with >3 fold increase over control.
* All particles tested showed a significant increase in induced necrosis from 50 µg/ml however this was the most marked for rod-shaped particles (>double NPs).
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| Three sizes of Fe2O3 particles(Freyria et al. 2012) | Fe2O3 nano: diameter 93 nm (TEM); SSA 19-22 m2/g.Fe2O3 submicro: diameter 260 nm (TEM); SSA 4-8 m2/g.Fe2O3 micro: diameter 1600 nm (TEM); SSA 13-14 m2/g.Exposure concentrations: up to 100 µg/cm2. | Synthesized: by precipitation of FeCl3 in HCl.  | Human lung epithelial cells (A549) and murine alveolar macrophages (MH-S). | * No significant cytotoxicity was seen in either cell line at concentrations up to 100 µg/cm2.
* Genotoxicity was not seen in either cell line at concentrations up to 100 µg/cm2.
* There was no hydroxyl radical detected even with the addition of ascorbic acid.
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| Nano Fe2O3 particles (Apopa et al. 2009) | Fe2O3 nano: <10 nm (supplier quoted); SSA 165 m2/g (supplier quoted).Exposure concentration: 0 – 50 µg/ml (no length of exposure indicated). | Purchased from chemical supplier (nGIMAT, USA). | Human microvascular endothelial cells (HMVEC). | * Showed rapid uptake into HMVECs.
* At concentrations as low as 12.5 µ g/ml caused a separation of cells, producing intercellular gaps and a decrease in transendothelial electrical resistance, both of which indicate compromise of the barrier and increased cell permeability via increased stabilisation of microtubules.
* No cell damage at any concentration tested (as identified through LDH assay).
* 50 µg/ml NPs led to an increase in ROS which was inhibited by catalase and only produced in cell culture, suggesting a cellular oxidative stress response.
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| Nano Fe2O3 particles(Rajiv et al. 2016) | Fe2O3 nano: 74.6 nm (average hydrodynamic diameter in water); 43.7 nm (TEM) spherical.Exposure concentration: 0 – 100 µg/ml for 24 hour exposure.  | Purchased from chemical supplier (Sigma-Aldrich). | Human lymphocytes isolated from peripheral blood. | * Significant decrease in cell viability for Fe2O3 for all concentrations > 25 µg/ml. At 100 µg/ml there was <50% cell viability.
* Fe2O3 was caused a significant increase in %LDH leakage at all concentrations tested (50-100 µg/ml) up to >50% at 100 µg/ml.
* Significant ROS generation at 50 and 100 µg/ml.
* Significant reduction in Catalase, reduced GSH and superoxide dismutase at 75 and 100 µg/ml (indicative of a decrease in anti-oxidants) and a significant increase in lipid peroxidation at all concentrations tested (>50 µg/ml).
* Tail DNA damage of 9.3% for 24 hr exposure to 100 µg/ml Fe2O3 NPs (SiO2 was only 3.4% and Co3O4 was 10.1%).
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| Nano Fe2O3 (hematite) particles(Bhattacharya et al. 2009) | Fe2O3 nano: ~50 nm (hydrodynamic diameter) spherical (SEM); Zeta -28.68 mV.Exposure concentration: 0 – 50 µg/ml for 6, 12, or 24 hour exposure. | Purchased from chemical supplier (Sigma-Aldrich). | Human lung fibroblasts (IMR-90) and human bronchial epithelial cells (BEAS-2B, SV40 virus transformed). | * Hematite NPs induced a concentration dependent loss of cell viability after 24 hours of exposure in both cell lines (p<=0.05).
* Fe2O3 NP exposure induced significant DNA damage at concentrations from 10 µg/cm2 in IMR-90 and 50 µg/cm2 in BEAS-2B cells. In the same study TiO2-NPs showed no DNA-breakage.
* Fe2O3 NPs produced lower ROS than TiO2, however when they were co-exposed to ascorbic acid and H2O2 or cell lysate and H2O2 they produced significant ROS suggesting that the Fe(II) required reducing conditions for radical formation.
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| Nano Fe2O3 particles(Stueckle et al. 2017) | Fe2O3: Diameter (BET) 11.9 nm; SSA = 96 m2/g. Exposure concentrations: 0.6 µg/cm2 twice a week for up to 12 weeks. | Flame synthesis: flame spray pyrolysis using the Harvard Versatile Engineered Nanomaterial Generating System (VENGES). | Human small airway epithelial cells (pSAECs) | * Subchronic exposure to Fe2O3 particles for 10 weeks at a delivered dose of 0.180 µg/cm2 resulted in a neoplastic-like transformation and replicative immortalization of human pSAECs at comparable levels to known engineered nano-material tumour promoter (MWCNT).
* The authors suggest that Fe2O3 exposure may be a risk factor for early neo-plastic like transformation in the human lung. Comparatively CeO2 particles showed enhanced proliferation but little evidence of neoplastic behaviour.
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| Nano Fe2O3 particles(Demokritou et al. 2010) | Fe2O3 nano: 4.7 nm; SSA ~300 m2/g.Fe2O3 nano: 6.7 nm; SSA ~200 m2/g.Exposure concentration: 50 and 500 µg/ml for 3 hour exposure. | Flame synthesis: iron (III) acetylacetonate: 2-ethyl hexanoic acid (1:1). | Alveolar macrophages – monocyte derived macrophages (AM-MDMs) | * Membrane integrity study showed no cytotoxicity at the lowest concentration (50 µg/ml) but showed significant cytotoxicity at a concentration of 500 µg/ml with 78.95% positive cells for the 4.7 nm particles and 60.00% positive cells for the 6.7 nm particles.
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| Nano Fe2O3 (both maghemite & hematite) particles(Park et al. 2014b) | Fe2O3 nano: ~102 nm (hydrodynamic diameter TEM); Zeta -28.97 mV (vehicle) and -7 mV (culture).Exposure concentration: 6.25 – 50 µg/ml for 24 hour exposure. | Synthesized: precipitated in alkali solution from Fe(II) and Fe(III) chloride following by subsequent oxidation to Fe2O3. | Mouse peritoneal macrophage cell line (RAW264.7) (high lysosomal activity) | * NPs were incorporated into the cells via pseudopodia, and were then located within autophagosome-like vacuoles with a small number of NPs located freely in the cytosol.
* There was also alteration of the mitochondria, endoplasmic reticulum and Golgi apparatus observed.
* There was a significant decrease in cell viability at all concentrations tested (from 6.25 µg/ml) which was concentration dependent down to 73.5% viability for 50 µg/ml.
* ATP production, mitochondrial calcium level and expression of ferritin all decreased on cell exposure to Fe2O3 NPs.
* The number of cells producing ROS increased in a dose dependent manner.
* Cytokine synthesis increased.
* There was little change in the number of cells in the subG1 phase which suggests that the cause of cell death was not apoptosis. Changes to cytosolic organelles and increased gene transcription of autophagic-related proteins suggests that cell death may be driven by autophagy via oxidative stress pathways.
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| Co-exposure to nano Fe2O3 (maghemite) particles and CB(Berg et al. 2010) | Fe2O3 nano: 41 nm (TEM); Zeta -44.2 mV; >99.99% purity by mass.Exposure concentration: 0 – 100 µg/ml (with 4 µg/ml CB) for 25 hour exposure. | Flame synthesis: H2/air diffusion flame seeded with Fe(CO)5 vapour. | Human lung epithelial cells (A549). | * TEM showed internalisation and co-localisation of the Fe2O3 NPs with engineered CB into sub-micrometre sized vesicles.
* Fe2O3 produced a significant amount of oxidant only at the highest concentration (100 µg/ml).
* However, oxidation is significant for all concentrations following co-exposure to Fe2O3 and CB with the highest oxidation seen for 0.01 µg/ml Fe2O3: 4 µg/ml CB with an increase of 104% over the control.
* Only low levels of cytotoxicity with the highest levels seen for 10 µg/ml Fe2O3: 4 µg/ml CB with 7.6% cell death, which can be directly compared to the 1.48% cell death seen for the same concentration where the CB surface was oxidized which will diminish its reductive capacity, therefore suggesting that it is the potential redox cycling between these two particles which causes toxicity.
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| Co-exposure to nano Fe2O3 (maghemite) particles and CB(Guo et al. 2009) | Fe2O3 nano: 41 (TEM); SSA 49 m2/g.Exposure concentration: 0 – 100 µg/ml (with 1-100 µg/ml CB) for 25 hour exposure. | Flame synthesis: H2/air diffusion flame seeded with Fe(CO)5 vapour. | Human lung epithelial cells (A549) | * Neither Fe2O3 nor CB alone showed a significant level of protein oxidation relative to the control, however, cells co-exposed to CB and Fe2O3 showed significant protein oxidation for 5 of the 10 concentration ratios tested.
* The trend was not linear with increasing concentration of either particulate.
* Exposure to either CB or Fe2O3 NPs alone failed to show significant lipid peroxidation but co-exposure to 0.1 µg/ml Fe2O3: 4 µg/ml CB showed a level of lipid peroxidation significantly higher than the control.
* CB and Fe2O3 co-incubated with 0.75 M H2SO4, to replicate lysosomal conditions, produced both Fe3+ and Fe2+ dissolved in solution. The concentration of the reduced iron increased with incubation time. This can be compared to Fe2O3 which when incubated under the same conditions produced only Fe3+.
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| Coated (carboxydextran) SPION (Chen et al. 2010) | Ferucarbotran (SPION): 60-68 nm (DLS); Zeta -21 to -27 mV (conc. dependant).Exposure concentration: 0 – 300 µg/ml for 1 hour exposure. | Purchased from chemical supplier (Bayer Schering Pharma, Germany). | Human mesenchymal stem cells (hMSC). | * Caused a dose dependent decrease in ALP production (which is an indicator of osteogenic differentiation), significant from 10 µg/ml.
* Promoted cell migration, as evidenced by the increase in protein expression of matrix metalloproteinase 2.
* Desferrioxamine, an iron chelator, antagonized the osteogenic differentiation inhibition, suggesting that free iron was responsible for the toxicity.
* Cell free analysis determined release of 0.0007% of iron into media (1 hour).
* Free iron was likely released by lysosomal degradation of Ferucarbotran.
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| Coated (TWEEN) SPION(Naqvi et al. 2010) | SPION: 30 nm mean diameter (DLS); spherical shape (TEM).Exposure concentration: 0 – 500 µg/ml for 3 or 6 hour exposure. | Synthesized: Tween 80, ferrous sulphate and NaOH, partially oxidized then precipitate dried. | Mouse macrophage cells (J774). | * SPIONs were incorporated into the cells via endocytosis.
* Showed a time and concentration dependent cytotoxicity.
* At concentrations ≥200 µg/ml incubated for 6 hours cell viability decreased to 55-65%.
* Oxidative stress induced by SPION was shown to be time-dependent.
* Apoptosis of cells was shown to be time and concentration dependent and likely related to cellular oxidative stress.
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| Nanozerovalent iron particles (Sun et al. 2016) | Nanozerovalent iron particles: irregularly shaped; SSA 40 m2/g; average hydrodynamic diameter 57.1 nm; Zeta potential in medium -24.8 mV.Exposure concentrations: 0 – 800 µg/ml for 24 hour exposure. | Synthesized: from NaBH4 and FeCl3 | Human lung epithelial cells (A549) and endothelial human cell line (EA.hy926) | * Concentration dependent cytotoxicity in A549 cells at concentrations ≥ 50 µg/ml for 24 hours with an EC50 of 359.0 µg/ml.
* Co-culture of particles with A549 and EA.hy926 endothelial cells (at 10 µg/ml) showed exclusive uptake in A549 lysosomes with no uptake in endothelial cells.
* Significant increases were seen in cell death of both A549 and EA.hy926 cells in co-culture with increases in LDH, death was likely due to apoptosis.
* Oxidative stress was evident in both cell lines but 8-OHdG increase was only observed in EA.hy926 cells.
* Significant increase in IL-6 and IL-1β in both cell lines after 10 µg/ml exposure. E-selectin, ICAM and IL-8 were also significantly elevated in EA.hy926 cells.
* Authors conclude that in this co-culture system nanozerovalent iron at low levels (10 µg/ml) has the potential to cause adverse oxidative responses in the lungs.
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**Table SM2: Summary of *in vitro* studies involving exposure to iron containing particulate matter. Abbreviations: GMA – gas metal arc; IL – interleukin; LDH – lactate dehydrogenase; MMA – manual metal arc; MN – micronuclei; MS – mild steel; PAH – polycyclic aromatic hydrocarbons; PM – particulate matter; ROS – reactive oxygen species; SS – stainless steel; TNF – tumour necrosis factor.**

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| **Particle Type and Reference** | **Particle Properties, Size, and Concentrations** | **Particle Source** | **Cell Type** | **Toxicity results** |
| Subway particles(Jung et al. 2012) | Subway particles PM10: no determination of Fe content or size distribution of PM.Exposure concentration: Organic extract of PM at 0 – 100 µg/ml for 24 hour exposure. | Natural source: collected from South Korean subway station (Kil-eum subway station). | Chinese hamster ovary cells (CHO-K1) and Human lung endothelial cells (BEAS-2B) | * No cytotoxicity was seen in BEAS-2B cells but CHO-1 cells showed a concentration dependent cytotoxicity with all concentration ≥25 µg/ml showing a significant reduction in cell viability.
* Genotoxicity in CHO-1 was determined. MN frequency increased significantly over the control at all concentrations ≥1.6 µg/ml and without rat liver homogenate at all concentrations ≥ 6.3 µg/ml.
* MN formation was significantly reduced when the same assay was run in the presence of ROS scavengers suggesting that the mechanism likely involved ROS production.
* A comet assay on CHO-K1 cells showed an increase in DNA breakage at all concentration ≥1.6 µg/ml.
* BEAS-2B cells showed an increase in MN formation at concentrations ≥1.6 µg/ml and the comet assay on these cells showed an increased DNA breakage at concentrations ≥ 6.3 µg/ml.
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| Subway particles(Loxham et al. 2015) | PM was 32% iron (coarse); 28% iron (fine); and 33% iron (ultrafine) by mass.Collected PM concentrations: 180 mg/m3 (coarse), 71 mg/m3 (fine), and 44 mg/m3 (ultrafine). Exposure concentrations: 0, 5, 10, 25 and 50 µg/ml for 24 hour exposure. | Natural source: collected from European mainline underground railway station. Particles collected and separated into PM10-2.5 (coarse); PM2.5 (fine); and PM0.18 (ultrafine) suspensions in ultrapure water. | Primary bronchial epithelial cells (PBECS) collected from severe asthmatics and controls. | * Healthy donor cultures showed a concentration dependent increase in IL-8 release for all three sizes. With a further trend toward an increased upper bound of IL-8 release with increased particle concentration suggesting significant variation in response between individuals.
* In asthmatic donor cells the concentration dependence was starker for coarse and fine particles but less defined for ultrafine with 50 µg/ml fine particles producing a significant increase in IL-8 in asthmatic donor cells.
* There was no significant LDH release at any concentration for any particle size in healthy or asthmatic donor cells.
* There was a concentration dependent increase in ROS, this increase was significant from 3.1 µg/cm2 for all size fractions.
* The ultrafine fraction was tested in the presence of an iron chelator (DFX) which caused a concentration dependent decrease in ultrafine PM induced ROS formation suggesting the ROS generation is caused by the iron content in PM.
* The cells also upregulated their expression of the anti-oxidant enzyme HO-1 following 8 hr exposure to 5.6 µg/cm2 of ultrafine PM with a 4.6 fold HO-1 expression increase (P<0.001).
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| Motor vehicle brakewear particles.(Puisney et al. 2018) | Particle size 200 nm – 10 µm (median 1.47 µm)29.1% FeExposure concentration: 1 – 100 µg/cm2 for 24 hour exposure. | Natural source: Motor vehicle brakewear particles (BWP). Sonicated and filtered to separate the nano fraction | Human lung epithelial cells (Calu-3). | * WST-1 mitochondrial activity assay significant following exposure to total size fraction BWP from 1 µg/cm2 with viability reduced by 81% at 100 µg/cm2.
* Significant from 5 µg/cm2 for the NP component of BWP but this only had a 35% decrease in viability at 100 µg/cm2.
* Both size fractions showed an approximate doubling in ROS production at 100 µg/cm2 following a 4 hour exposure.
* BWP, IL-8 and TNFα were not significantly altered following exposure.
* IL-6 showed a significant increase from 10 µg/cm2 for BWPs with a 5-fold increase at 50 µg/cm2.
* The nano fraction produced significantly less IL-6. From this study it appears the nano-fraction of BWP is less toxic than BWPs, authors suggest this might be more to do with actual delivered dose to the cell of the different particle sizes than an actual difference in toxicity.
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| Printing toner(Gminski et al. 2011) | 3 different toner powders (A, B, C): slightly elongated shape, 30-200 nm diameter. XRD suggested the iron was present as magnetite. Toner A was 3% iron by weight, B was 23% iron by weight and C was 28% iron by weight. Exposure concentration: 0 – 400 µg/cm2 for 24 hour exposure. | Purchased from printing supplier: A (Kyocera TK-16H), B (Kyocera TK-17), and C(Hewlett-Packard LaserJet C4092A). | Human lung epithelial cells (A549). | * Iron was largely water soluble with >89% of the iron detected in the aqua regia digestion (A and C >97%).
* At concentrations up to 400 µg/cm2 there was virtually no decrease in cell viability. All powders produced statistically significant increases in DNA migration in a concentration dependent manner with toner B producing the largest increase (toner B has the highest conc. of PAHs).
* Induction was micronuclei was significant and concentration dependent for all three toners. Significant from 133 µg/cm2 for toners A and C and from 200 µg/cm2 for Toner B.
* Concluded that the PAHs are responsible for the genotoxic effects.
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| Welding fumes(Leonard et al. 2010) | SS welding fumes: 57.0% Fe, 20.2% Cr.MS welding fumes: 80.6% Fe, 14.7% Mn.Fumes formed large agglomerated chains of particles. Ultrafine particles: 0.01–0.056 µm.Fine particles: 0.10–1.0 µm.Coarse particles: 1.8–18 µm.Exposure concentration: 250 µg/ml or 500 µg/ml for 30 minute or 1 hour exposure. | Welding fumes generated in situ and collected on filters. SS – stainless steel. MS – mild steel. | Mouse peritoneal monocytes (RAW 264.7). | * Strong radical generation was observed by electron spin resonance for both the SS and MS 1 hour post fume formation.
* Older samples produced significantly less ROS.
* Addition of deferoxamine (a metal chelator) resulted in a decrease in radical signal strength. Addition of catalase also decreased the signal.
* When the same mass of each sample was examined SS showed a significantly higher generation of hydroxyl radicals than MS.
* SS fumes showed a significant increase in lipid peroxidation for all three sizes, where the MS was only significant for ultrafine particles.
* MS and SS fumes at all sizes produced a significant increase in H2O2 production with SS increasing significantly more than MS for fine and ultrafine particles.
* Comet assay showed a significant increase in the amount of DNA damage for both SS and MS with SS showing a significant increase over MS (250 µg/ml).
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| Welding fumes(Shoeb et al. 2017) | GMA-MS sample was 85% Fe. MMA-SS was only 44% Fe and had a higher soluble component.Exposure concentration: 0, 3.125,6.25, 12.5, 25, 50, or 100 µg/ml for 24 hour exposure. | Natural: welding fumes collected on filters, extracted and suspended in saline. | Mouse peritoneal monocytes (RAW 264.7). | * Dose dependent decrease in cell viability for both MS (significant from 25 µg/ml) and SS fumes (significant from 12.5 µg/ml).
* SS fumes also lead to a significant increase in Caspase-3/7, and cyclooxygenase-2 expression, where MS fumes were not significant.
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| MS Welding fumes(Suri et al. 2016) | MS fumes were 12.4% iron by weight.Exposure concentration: Exposed cells to fumes for 2 hours at 275 ug/ml for A549 and 200 ug/ml for BEAS-2B. | Natural: MS welding fumes collected on filters, extracted and stored until use. | Human lung epithelial cells (A549) and human lung endothelial cells (BEAS-2B) | * Oxidative potential of welding fumes for ascorbate and glutathione was increased c.f. carbon black.
* MS increased platelet-activating factor receptor protein expression in A549 and BEAS-2B cells, this was attenuated by N-acetylcysteine (anti-oxidant).
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| Urban PM, China(Ho et al. 2019) | Urban PM, Xi’an: average PM2.5 104.2 µg/m3.Urban PM Beijing: average PM2.5 85.7 µg/m3. | Urban PM, collected on filters and extracted.  | Human lung epithelial cells (A549). | * Fe from industrial sources showed a significant negative association with cell viability for all times and during dust storms from Xi’an but not vehicle sources from Beijing.
* Fe also showed a significant positive correlation with LDH for Fe from industry sources in Xi'an at all times and during dust storms from vehicle sources in Beijing.
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**Table SM3: Summary of *in vivo* studies involving iron oxide particles. Abbreviations: ALP – alkaline phosphatase; AM – alveolar macrophages; BALF – bronchoalveolar lavage fluid; DLS – dynamic light scattering; EPR – electron paramagnetic resonance; GSH – glutathione; IG – immunoglobulin; IL – interleukin; LDH – lactate dehydrogenase; MDA – malondialdehyde; NP – nanoparticles; ROS – reactive oxygen species; SEM – scanning electron microscopy; SPION – superparamagnetic iron oxide particles; SSA – specific surface area; TEM – transmission electron microscopy; TNF – tumour necrosis factor; XANES – X-ray absorption near-edge structure; Zeta – Zeta potential.**

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| **Particle Type and Reference** | **Particle Properties** | **Particle Source** | **Animal Model and Exposure Conditions** | **Toxicity Results** |
| Micro Fe2O3 particles in rats(Beck-Speier et al. 2009) | Fe2O3 micro small: 0.5 µm; SSA 17 m2/g.Fe2O3 micro large: 1.5 µm; SSA 7 m2/g. | Flame synthesis: Fe2(NO3)3 at 800 °C. | WKY rats, intratracheal instillation.Instilled dose: 1.3 mg/kg body weight for 0.5 µm particles; 4.0 mg/kg body weight for 1.5 µm particles. Euthanized and BALF extracted 24 hours post exposure. | * The larger particles induced neutrophil influx and increased vascular permeability.
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| Nano and submicron Fe2O3 particles instilled in mice(Wang et al. 2009) | Nano Fe2O3: 21 nmm (TEM).Submicron Fe2O3: 280 nm (TEM). | Purchased from chemical supplier: Nano (Nanjing Haitai Nanomaterial, China); submicron (Chengdu Shi-Jia-Wei-Er, China). | CD-ICR mice, intranasal instillation.Instilled dose: 130 µg every second day for duration of study. Mice were euthanized at 12, and 72 hours and 14 and 30 days. | * GSH-Px activities were significantly increased in the olfactory bulb and hippocampus following both size particles at 12 hours post-instillation but not in the cortex, cerebellum, or brain stem.
* GSH significantly decreased in the olfactory bulb and hippocampus following exposure to both sizes.
* Significant increase in T-nitric oxide synthase and c-nitric oxide synthase in the olfactory bulb, hippocampus and cerebellum for nano particles.
* Authors conclude that changes seen in the brain are due to particle translocation.
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| Nano Fe2O3 particles in rats, long-term inhalational study(Sutunkova et al. 2016) | Fe2O3 nano: 10-20 nm (Raman confirmed); spherical (SEM).  | Synthesized: sparking from 99.99% pure iron rods. | White rats, inhalation. Inhaled dose: ~1 mg/m3 4 hours/day, 5 days/week for 3, 6 or 10 months.Euthanized, BALF extracted and organs excised.  | * Significant increase in total cell count, particularly neutrophils and AM, in BALF.
* Some NPs seen (via TEM) in the olfactory region of the brain, possibly via translocation from the nasal cavity along the olfactory nerve fibres (note: concentration too low to be detected by EPR).
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| Nano Fe2O3 particles in rats(Demokritou et al. 2010) | Fe2O3 nano: 4.7 nm; SSA ~300 m2/g.Fe2O3 nano: 6.7 nm; SSA ~200 m2/g. | Flame synthesis: iron (III) acetylacetonate: 2-ethyl hexanoic acid (1:1). | Sprague-Dawley rats, intratracheal instillation.Instilled dose: 1 mg/kg body weight (1.5 ml volume/kg body weight).Euthanized and BALF extracted 24 hours post exposure. | * Showed significant BALF parameters of inflammation including a significantly increased level of neutrophils, but not macrophages, lymphocytes or eosinophils
* As well as in increase in LDH (measure of cell membrane disruption), myeloperoxidase (indicator of neutrophil degranulation) and albumin (measure of capillary permeability) but no increase in haemoglobin.
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| Radiolabelled Fe2O3 instilled in rats to determine distribution and kinetics.(Zhu et al. 2009) | Radiolabelled α-59Fe2O3 nano: 144 nm hydrodynamic diameter (DLS); SSA 53.27 m2/g. | Purchased from chemical supplier (Nanjing Haitai Nanomaterial Co. Ltd, China). | Sprague-Dawley rats, tracheal instillation.Instilled dose: 4 mg/rat 59Fe2O3.Blood samples, urine and faeces collected 10 mins – 50 days post instillation.Euthanized and organs excised 1, 7, 21, or 50 days post instillation. | * The particles had an extremely long elimination half-life (22.8 days) and a limited lung clearance rate (-3.06 μg/day) with 86.94 % of iron found in organs still located in the lungs 50 days post instillation.
* Slow elimination leads to a higher risk of cumulative exposure effects.
* Majority of the iron was excreted in faeces (likely swallowed after elimination from lung).
* Particles passed through the alveolar-capillary barrier into the systemic circulation in < 10 min.
* Distribution to organs rich in mononuclear phagocytes such as liver, spleen, kidney and testicles.
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| Nano Fe2O3 in rats, nasal instillation study(Askri et al. 2018) | Fe2O3 nano: 30 nm diameter, spherical shape (TEM).  | Synthesized: from iron(III) acetylacetonate dried under supercritical ethanol.  | Wistar rats, intranasal instillation.Instilled dose: 10 g/kg body weight each day for 7 days.Euthanized 7 days post exposure. | * Fe2O3 exposed rats had a significantly higher body weight from day 4-8 than control rats.
* Exposure significantly decreased the weight of the kidneys and liver compared to the controls but not the brain, spleen or lungs.
* No significant difference was seen in the anxiety index or Morris water maze test, suggested no alteration to behavioural performances in rats.
* Significant increase in platelet count but no changes to white blood cell, red blood cell, haemoglobin or %haematocrit.
* Significant decrease in alkaline phosphatase and iron in blood as well as a significant increase in dopamine and norephinephrine likely caused by stress induced response.
* Interestingly there was no increase in iron concentration in the lung of exposed rats.
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| Nano Fe2O3 in mice, intratracheal instillation study(Ban et al. 2012) | Fe2O3 nano: 35 nm diameter; SSA 39 m2/g.Fe2O3 submicron: 147 nm diameter; SSA 6 m2/g | Purchased from chemical supplier (Sigma Aldrich) | BALB/c mice, intratracheal instillation.Instilled dose: single instillation of 250 µg, 375 µg or 500 µg per mouse OR 4 x 500 µg instillations over 12 days.Euthanized at various timepoints. | * Significant increase in the total number of cells for all treatment protocols for both sized particles (1.2 - 2.6 fold increase) with the largest increase (2.6 fold) seen for 375 µg NPs.
* Significant increase in neutrophils for all protocols and both particle sizes. For the 375 µg nanoparticle dose ~40% of the total BAL was neutrophils.
* Increase in IL-2, IL-6 and interferon-γ in lymph node cell culture for high single dose administration but not for the 4 dose protocol.
* High dose submicron iron oxide and all doses of NPs caused a significant decrease in lymphocyte plaque forming cells indicating a decrease in humoral immunity in lung lymph nodes. However, decreases were seen for some inflammatory markers in BAL the authors suggest that 48 hours post instillation might not be an appropriate time analysis for these markers.
* Both sizes of particle induced an inflammatory reaction in the lung and local immunosuppression.
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| Nano Fe2O3 (hematite) particles in mice(Gustafsson et al. 2015) | α-Fe2O3 particles: 30 nm diameter (TEM), SSA 28.98 m2/g. | Purchased from chemical supplier (not provided). | Balb/c mice, intratracheal instillation. Instilled dose: 0, 1.25, 2.5, or 5.0 mg/kg.Euthanized at various timepoints. | * Significant increase in total leukocytes at 5.0 mg/kg dose and neutrophils from 2.5 mg/k dose 1 day after dose.
* Following a 2.5 mg/kg dose total leukocytes were highest 1 day post instillation, neutrophils were highest 1 day post instillation, lymphocytes were highest 2 days post instillation and macrophages were highest 7 days post instillation. All of these were a significant increase over control (p<0.01).
* Mice with an induced allergic airway disease responded differently to iron oxide particle exposure.
* Mice with already established inflammation had a reduction in inflammatory cells following iron oxide exposure.
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| Nano maghemite and hematite particles instilled in mice(Wang et al. 2011) | α-Fe2O3 nano: 22 nm; SSA 11.18 m2/g; -10.5 mV.γ-Fe2O3 nano: 31 nm; SSA 33.21 m2g; -5.3 mV. | Purchased from chemical supplier (α-Fe2O3 Nanjing Haitai Nanomaterial Co. Ltd, China; γ-Fe2O3 Sigma-Aldrich, USA). | CD-ICR mice, intranasal instillation.Dose instilled: 130 µg every other day for 40 days.Euthanized and brain removed post final instillation. | * Significant increase in Fe in the olfactory nerve layer, glomerular layer, external plexiform layer, internal plexiform layer and anterior olfactory nucleus external part of the olfactory bulb.
* Fe also significantly increased in the cortex, hippocampus and midbrain following NP instillational exposure.
* Neuropathological changes to the mouse brain such as irregular arrangement of neuron cells or neuronal loss in olfactory bulb as well as neuropathological changes in the hippocampus and striatum.
* Increased number of activated microglia in olfactory bulb of treated mice.
* *In vitro* BV2 cells exposed to NPs. No significant cytotoxicity. Induced significant excretion of ROS and nitric oxide.
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| Nano Fe2O3 particles in mice(Park et al. 2015) | γ-Fe2O3 nano: Needle-like NPs 50-200 nm, 10 nm width (TEM).Zeta (varied with solvent used) 11.9 mV (PBS) to -219.1 mV (Gamble’s solution). | Synthesized: precipitated in alkali solution from Fe(II) and Fe(III) chloride. | ICR mice, tracheal instillation.Instilled dose: 0 – 2.0 mg/kg.BALF extraction and blood collection on day 90. | * On day 90 significant increases were seen for total BALF cell count for all concentrations (lowest 0.5 mg/kg) and neutrophils and lymphocytes were significantly increased for the 2.0 mg/kg group.
* There was a significant increase in cytokine concentration for all cytokines tested, the most marked being an 8.7 fold increase in IL-12 for the 2.0 mg/kg dose, with particular increase in IgG and IgM but not IgE.
* There was a significant elevation in LDH release into the lung for the 1.0 and 2.0 mg/kg doses.
* There was a significant increase in the expression of Chitinase 3-like 1 gene at the 2.0 mg/kg dose. The equivalent gene in humans (YKL-40) has been implicated in airway remodelling, cancer progression and activation of the anti-apoptotic signalling pathway.
* The proportion of cells in the subG1 region of the cell cycle (which is representative of apoptotic cell death) increased with particle concentration, with the 2.0 mg/kg dose having a 6 fold increase in apoptotic cells.
* Comparing with earlier papers by the same research group they concluded that the needle like iron particles were more toxic than the equivalent spherical particles.
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| Soot and nano Fe2O3 particle inhalation in rats.(Zhong et al. 2010) | Fe2O3 nano: 72 nm (TEM); Fe2O3 determined by Fe:O ratio.  | Flame synthesis: ethylene/acetylene passed over Fe(CO)5 and burnt in flame (soot and iron oxide controlled independently). | Sprague-Dawley rats (10 + 23 day old), whole-body inhalation.Inhaled dose: total particle concentration 250 µg/m3; Fe2O3 concentration 30-100 µg/m3; 6 hours/day for 3 days.Euthanized and BALF collected 24 hours post inhalation. | * Significant decrease in cell viability and increase in LDH activity (cell necrosis).
* Significant (2.5 fold) increase in ferritin expression (indicative of an increase in bioavailable Fe) as well as a significant increase in IL-1β.
* Exposure to both 30 and 100 µg/m3 Fe-containing particles in combination with soot caused a significant increase in oxidized glutathione and glutathione redox ratio (indicating an increase in oxidative stress).
* Concluded that a combination of Fe and soot led to an increase in lung injury, oxidative stress and inflammatory response in neonatal rats.
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| Co-exposure to nano Fe2O3 and soot particles in mice(Hopkins et al. 2018) | Fe2O3: Mean particle size 50 nm | Flame synthesis: ethylene/acetylene passed over Fe(CO)5 and burnt in flame | C57B6 mice, inhalation via whole body exposure.Iron-soot ultrafine concentration of 200 µg/m3 total, with 40 µg/m3 Fe2O3, 6 hr/day for a total of 25 days. | * Ultrafine iron oxide particles reached the brain via olfactory nerve fascicles following inhalation.
* Total number of microglial cells did not change with iron-soot exposure. However, the ratio of activated to resting microglial cells was significantly increased following iron-soot exposure and the level of inflammatory cytokine IL-1β in the olfactory bulb was also significantly increased.
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| Nano Fe3O4 particles in rats, short-term inhalational study(Srinivas et al. 2012) | Fe3O4 nano: 48 nm (SEM); hydrodynamic diameter 651.2 nm; Zeta -15.9 (mV); SSA ≥40 m2/g. | Purchased from chemical supplier (Nanostructured and Amorphous Materials Inc). | Wistar rats, inhalation.Inhaled dose: 640 mg/m3 for 4 hours.Euthanized, blood collected and BALF extracted 1, 2 or 14 days post inhalation. | * Blood analysis showed no significant changes for any biochemical markers tested including erythrocytes and haemoglobin.
* Significant decrease in cell viability in BALF at all post-inhalational time points. Most significant at 1 day post inhalation with ~60% cell viability compared to control.
* Significant increase in BALF cell count, 2.5 fold increase at 1 day post inhalation, neutrophils had a 5-fold increase at 1 day followed by time-dependent decrease.
* LDH (marker of cell damage via necrosis), total protein (indicator of alveolar epithelial-capillary barrier injury) and ALP (enzyme leakage) were all significantly increased throughout the 14-day test period.
* Concentrations of pro-inflammatory cytokines were increased up to 20-fold.
* Significant increase in malondialdehyde (indicative of lipid oxidation and therefore ROS generation) with time-dependent recovery
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| Nano Fe3O4 particles in rats.(Szalay et al. 2012) | Fe3O4 nano: < 50 nm; SSA >60 m2/g (supplier quoted). | Purchased from chemical supplier (Sigma-Aldrich, USA). | Wistar rat, tracheal instillation.Instillation dose: low dose - 1 mg/kg body weight; high dose - 5 mg/kg body weight.Euthanized 1, 3, 7, 14 or 30 days after treatment. | * General and organ toxicity was seen in vivo with decreased total body weight and a significant decrease in lung weight likely to be caused by oxidative stress.
* At 7 days kidney and liver weights were also significantly lower than the control but these had a time-dependent recovery.
* Pathological examination identified a weak pulmonary fibrosis and interstitial inflammation had developed in the lungs which was more substantial in the high dose rats.
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| Fe3O4 inhalation in rats.(Pauluhn 2012) | Fe3O4: Average pore diameter 981 nm; SSA 10 m2/g.Iron content was 69.5%. | Purchased from chemical supplier (Rockwood, Italy). | Wistar rats, nose-only inhalation.Inhaled dose: 1, 4.7, 16.6 and 52.1 mg/m3 for 6 hr/day, 5 day/week, 13 weeks. | * There was no mortality or systemic toxicity observed.
* Slight increase in neutrophils in blood with a larger increase in BAL.
* Pulmonary inflammation was evident from BAL changes and histopathology.
* Increased septal collagenous fibres were observed at the highest exposure concentration.
* Author state that all observations can be attributed to a response to poorly soluble particle exposure not to the direct effect of soluble iron.
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| Fe3O4 nanoparticles in instilled mice(Totsuka et al. 2014) | Fe3O4: 10 nm (TEM)’ spherical; Zeta potential of 30-40 mV at pH 10. | Purchased from chemical supplier (Toda Industrial, Japan). | ICR mice and gpt delta mice, intratracheal instillation. Instilled dose: 0.05 or 0.2 mg/animal for a single instillation an instillation each week for 4 weeks. | * Significant increase in DNA damage (via tail moment) in ICR mice (sacrificed 3 hours after instillation).
* Increase in gpt mutation frequency following 4 instillations (significant at 4 x 0.2 mg).
* Significant increase in 8-oxodG adducts in iron particle treated mice.
* Authors conclude that nanomagnetite is genotoxic and mutagenic in mice. Suggest that inflammatory responses lead to oxidative lipid peroxide related DNA adduct formations.
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| Nano Fe3O4 radiolabelled particles instilled in rats(Wu et al. 2013) | Fe3O4: diameter 30 nm (TEM); Zeta potential -9.1 mV for particles and -8.4 mV for labelled particles in physiological saline. | Purchased from chemical supplier (Sigma Aldrich) then radiolabelled by adding an amino group then reacting with Na125I. | SD rats, intranasal instillation.Instilled dose: instillation for either 1 day or 7 days and euthanised straight after instillation or 1 week or 2 weeks after completion | * The nanoparticles were deposited from highest to lowest concentration in the olfactory bulb, striatum, hippocampus, brain stem, cerebellum and frontal cortex.
* Clearance of particles was slow with more than half the iron particles in some brain regions still present 14 days post instillation.
* There were significant changes to oxidative damage markers (H2O2, superoxide dismutase and MDA) and striatum GSH levels 7 days after instillation.
* No significant changes in the number of brain lesions during the test period.
* Authors conclude that Fe3O4 particles were deposited and retained in the striatum after intranasal instillation.
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| Fe3O4 instillation in rats(Katsnelson et al. 2012) | Fe3O4: no data on size, SSA or Zeta potential. | Synthesized: from Fe(II) and Fe(III) chloride salts, precipitated using NH4OH. Polyacrylic acid (PAA) added for coated particles. | Albino rats, intratracheal instillation is deionized water (as the samples aggregated too quickly in physiological solution)Instilled dose: 2 mg/rat.BAL fluid collection. | * Significant number of particles taken up into alveolar macrophages (AM). Evidence of small phagosomes merging into larger phagosomes as well as free NP aggregates in the cytoplasm in close contact with membranes of organelles, particularly mitochondria, and the nuclear membrane.
* Electron microscopy identified a large number of destroyed AM which had released their NPs into the intercellular space.
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| Nano Fe3O4 particles in mice(Park et al. 2010) | Fe3O4: 5.3 nm (magnetically confirmed as Fe3O4). Surface charge 23.14 mV. | Synthesized: precipitated in alkali solution from Fe(II) and Fe(III) chloride. | ICR mice, tracheal instillation.Instilled dose: 0 – 1.0 mg/kg.Euthanized and BALF extracted on day 1, 7, 14 or 28 post instillation. | * There was an increase in cellular apoptosis, as indicated by an increase in cells in the subG1 phase (1.56% for the control compared with 6.96% 1 day after instillation).
* Significant increase in pro-inflammatory cytokines. Marked increase in IL-6 (day 1) and time dependent increase in IL-4 and IL-5 (to 28 days).
* Significant increase in IgE (antioxidant antibody) at day 1 in blood but not BALF.
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| Nano Fe3O4 particles in mice, short-term inhalation study(Teeguarden et al. 2014) | Fe3O4 nano: 12.8 nm (SEM); magnetite (TEM confirmed). | Synthesized: Co-precipitation of FeCl3 and FeSO4.7H2O in alkaline solution (NH3), magnetically collected.  | Balb/c mice, inhalation.Inhaled dose: 19.9 mg/m3 for 4 hours.Euthanized 0, 6, 24, 48, 96 hours or 7 days post exposure. | * Gave 365 µg inhaled and 14.5 µg deposited in lung (estimated).
* This level of exposure resulted in interstitial inflammation and macrophage infiltration with microscopic lung lesions.
* 815 genes were significantly changed followed inhalational exposure to SPION compared to the control.
* Up-regulated genes were mostly those involved in the immune response. Most of the up-regulated genes returned to base-line levels within 48 hours, however the down-regulated genes were still altered at 7-days post exposure.
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| Fe2O3 and Fe3O4 nanoparticles in mice(Presume et al. 2016) | Fe3O4 & Fe2O3: no data on size, SSA or Zeta potential. | Synthesized: in aqueous solution using the micro-waved-assisted sol–gel method. | C57BL/6 mice, pharyngeal aspiration.Aspirated dose: 5 or 50 µg µg per week for up to 3 months (estimated occupational unintentional exposure c.f 2.7 mg per 70 kg worker per day). | * At 5 µg per week for 3 months the two iron NPs did not show significantly different histology of lung tissue.
* The mice exposed to 50 µg doses however showed a significant increase in alveolar wall thickness, peribronchiolar wall thickness, perivascular wall thickness and interstitial lung inflammation for Fe3O4 NPs but not Fe2O3 NPs.
* All NPs at this dose showed pronounced lung fibrotic remodelling. Granuloma formation and development of emphysema were not observed.
* There was a significant increase in TNFα for 50 µg/week for 1 month Fe3O4 NPs. But no significant increase in total protein, LDH, transforming growth factor-β, number of cells in BALF or IL1β at either concentration.
* TNFα mRNA expression was increased in response to all NPs after 3 months of weekly exposure to 5 µg.
* Study suggested Fe2O3 NPs were less reactive than Fe3O4 NPs.
 |
| Fe3O4 and FeCO3 inhalation in rats(Pauluhn and Wiemann 2011) | Fe3O4: diameter 1.5 µm, SSA 9.5 m2/g.FeCO3: diameter 6.8/1.6 µm (elongated), SSA 5.6 m2/g. FeCO3 was significantly more water soluble. | Fe3O4 purchased from chemical supplier (Rockwood, Italy); FeCO3 purchased from chemical supplier (Sigma-Aldrich, Germany). | Wistar rats, nose-only inhalation.Inhaled dose: 32 and 100 mg/m3 for 6 hr/day, 5 day/week, 4 weeks with a 3 month post-exposure window for analysis (dose predicted to exceed overload threshold). | * Exposures were tolerated without any signs of toxicity, mortality or significant changes in body weight.
* Concentration dependent increase in total BAL cells particularly PMN.
* Concludes that the inflammatory response was dependent on total particle mass or volume and not on iron concentration.
* Non-specific pulmonary inflammation in rats is related to overload of non-soluble particles not iron-related ROS inflammation.
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**Table SM4: Summary of *in vivo* studies involving exposure to iron containing particulate matter. Abbreviations: 8-OH-dG – 8-hydroxydeoxyguanosine; 8-OHG – 8-hydroxyguanosine; BALF – bronchoalveolar lavage fluid; BMI – body mass index; CCL – chemokine ligand; ET-1 – endothelian-1; FCAW – flux-cored arc welding; FENO – fractional exhaled nitric oxide; GMAW – gas metal arc welding; ICAM-1 – intercellular adhesion molecule-1; IG – immunoglobulin; IL – interleukin; JNO – bronchial flux; LDH – lactate dehydrogenase; MCA – 3-methylcholanthrene; MDA – malondialdehyde; MMA – manual metal arc; MS – mild steel; NOS – nitric oxide synthase; PM – particulate matter; ROS – reactive oxygen species; SEM – scanning electron microscopy; SMAW – shielded metal arc welding; SS – stainless steel; SSA – specific surface area; TIG – tungsten inert gas welding; TNF – tumour necrosis factor; UFP – ultra fine particle; VCAM-1 – vascular adhesion molecule-1.**

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| --- | --- | --- | --- | --- |
| **Particulate Matter Type and Reference** | **Particulate Matter Properties** | **Particulate Matter Source** | **Animal Model and Exposure Conditions** | **Toxicity Results** |
| Occupational exposure, steel processing workers, human workplace exposure.(Tarantini et al. 2013) | Steel processing PM: No PM size or property details provided. | Natural: workplace exposure to PM from steel processing. | Human, ambient workplace PM exposure; N=63 Steel production workers, Italy.Exposure levels: PM10 233.4 ug/m3, PM1 8.5 ug/m3, iron 32.0 ug/m3. | * PM10 (-0.2, 95% CI -0.034 to -0.03) and PM1 (-0.7 95% CI -1.3 to -0.1) were significantly associated with a decrease in NOS3 methylation. Iron showed a borderline significant association with decrease in NOS3 methylation (-0.7 95% CI -1.4 to -0.01).
* PM10 (20.0 95% CI 3.0 to 37.0) and PM1 (80.8 95% CI 14.9 to 146.7) were positively and significantly associated with endogenous thrombin potential even after adjustment for age, BMI, smoking status and percent monocytes. Iron showed the highest regression coefficient for any of the metals analysed against endogenous thrombin potential (53.6 95% CI -16.5 to 123.7) but was not significant.
* Despite significant results for iron it is largely ignored in the discussion of this paper with focus on general PM and zinc. No mediation analysis shown for iron.
* Conclusions link thrombin generation (a marker for global coagulation) with DNA hypomethylation caused by metal containing PM among steel factory workers.
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| Occupational exposure, foundry PM, human workplace exposure(Liu et al. 2009) | Foundry PM: No PM property details provided. | Natural: workplace exposure. Foundry, Taiwan.  | Human, foundry workplace exposure. N=41 exposed foundry workers, 27 controls. Exposure levels: 0.99 mg/m3; 83.03 µg/m3 Fe; other metals (Al, Mg, Mn, Zn) all < 8 µg/m3. | * MDA (4.28 µM), 8-OH-dG (5.00 µg/g creatinine) and tail moment (Comet assay) (6.63) were all significantly higher in foundry exposed workers than administration controls (p<=0.001).
* Tail moment (Comet assay)-DNA strand breakage; MDA-oxidative stress marker.
* Despite the high levels of Fe this metal wasn't further analysed for risk factors associated with morbidity and mortality.
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| Occupational metal PM exposure in humans (Ahmed et al. 2013) | Iron industry PM: No PM size or property details provided. | Natural: workplace exposure to PM from steel processing. | Human iron industry workers, inhalational exposure.Exposure levels: levels not determined.Induced sputum. | * Cytological atypia was detected in 5% of iron cases and no controls (RR=17.5556 95% CI 0.9568-322.1004 P=0.0536).
* Squamous metaplasia was detected in 17.5% of iron cases but only 6% of controls (RR=2.7475 95% CI 1.2778-5.9076 P=0.0097) which is statistically significant.
* Significant increase in cytological evidence of viral infection with 21.3% of iron exposure cases but only 5% of controls showing evidence (RR=4.1703 95%CI 1.8813-9.2446 P = 0.0004).
* Significant increase in monilasis (yeast infection) in 30% of iron exposure cases but only 12% of controls (RR=2.4789 95% CI 1.4471-4.2467 P=0.0009).
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| Occupational metal PM exposure in humans(Cantone et al. 2011) | Iron industry PM: No PM size or property details provided. | Natural: workplace exposure to PM from steel processing. | Human iron industry workers, inhalational exposure, N=63.Exposure levels: determined at 11 locations in the workplace analysed against a workplace log.Whole blood analysis. | * H3K4me2 increased in association with iron (as well as nickel and arsenic) in an adjusted model.
* H3K9ac was positively but not significantly associated with iron levels.
* Histone modifications may indicate carcinogenic potential of toxic metals.
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| Occupational exposure to iron oxide(Ryu et al. 2013) | Mean exposure to iron oxide dust was 2.94 mg/m3 (arithmetic mean). | Natural: workplace exposure to organic solvents, exposure to iron oxide and exposure to welding fumes. | Human workers employed as ship builders or in chemical or metal production companies.N=448 (exposed).Spirometric test to determine obstructive pulmonary function impairment (OPFI). | * Prevalence of spirometric OPFI was highest among those exposed to iron oxide but this was not significant.
* Iron oxide exposure was highly associated with obstructive pulmonary function impairment (adjusted OR = 9.61; 95%CI 2.20-41.97).
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| Welding PM, human workplace exposure (Andujar et al. 2014) | Welding fumes: No PM property details provided. | Natural: workplace exposure. French welders, industries including shipbuilding (53%),automotive (14%), and sheet metal work (24%). | Human welders, inhalational exposure.Exposure levels: levels not determined.Collected non-tumour lung tissue samples against matched controls. | * Significant overload of Fe, Mn and Cr in welders lungs compared to control patients. Increased metal oxide loads were localised to the fibrous tissue in the lung.
* Siderophages, ferriginous bodies, fibrotic lesions and severity of fibrosis were all significantly higher welders compared to controls. Expression of cytokines IL-1β, TNF-α and CCL-3 were all significantly increased in lung tissue of welders compared to controls.
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| Welding PM, human workplace exposure(Pesch et al. 2015) | Welding fumes: No PM property details provided. | Natural: workplace exposure. Variety of workplaces, Germany. GMAW & FCAW on MS and SS; TIG; SMAW. | Human welders, inhalational exposure. Exposure levels: range of PM and Fe exposures. Median total welding fumes 0.94 mg/m3; median respirable Fe 201 µg/m3; individual levels measured on each worker.Blood and urine collected. | * There was a highly significant association between urinary 8-OH-dG and 8-OHG which are both markers of oxidation and serum ferritin which indicates high levels of iron.
* There were higher urinary 8-OHG concentrations in welders exposed to Fe above ~1 mg/m3. Authors suggest these findings suggest a role for iron-overload in oxidatively derived damage to guanosine from DNA metabolism in welders exposed to high Fe concentrations by inhalation.
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| Welding PM fumes, human workplace exposure (WELDOX study)(Casjens et al. 2014) | Welding fumes: No PM property details provided.  | Natural: workplace exposure. 25 German plants. | Human welders, exposed to a variety of welding fumes, N=192.Exposure levels: individually determined by personal exposure device.Whole blood analysis. | * Serum ferritin >300 ug/L in 23.8% of non-tungsten welders not wearing a respirator, c.f. 7.7% for those wearing a respirator and 9.2% for tungsten welders.
* Increased concentrations of serrum ferritin and prohepcidin in conditions with high respirable iron exposure (>1800 ug/m3).
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| Occupational exposure to welding fumes, human asthmatic exposure(Kauppi et al. 2015) | Welding fumes: No PM property details provided. | Natural: workplace exposure to welding fumes (average 27.5 years welding). | Human welders with diagnosed occupational asthma (taken off asthma meds for duration of study). N=16.Exposure levels: not determined.Lung function measurement and whole blood analysis. | * Blood leukocytes and neutrophils (blood) were both significantly increased following both MS and SS welding exposure.
* Blood platelets increased significantly following both MS and SS welding fume exposure.
* Hemoglobin and erythrocyte levels decreased significantly following both MS and SS exposure.
* These results suggest a mild systemic inflammatory response following welding fume exposure. This response appears to be time-limited as 22 hours after the last exposure levels had returned to baseline.
* There were no significant changes to the concentrations of any of the proinflammatory cytokines.
* The responses to SS fumes were more significant but the concentration of particles in the SS fume was also significantly higher.
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| Welding fumes instilled in rats(Shoeb et al. 2017) | GMA-MS sample was 85% Fe. MMA-SS was only 44% Fe and had a higher soluble component | Natural: welding fumes collected on filters, extracted and suspended in saline. | Sprague-Dawley rats, intratracheal instillation. Exposure levels: 2.0 mg/rat GMA-MS or MMA-SS welding fumes.Euthanized rats on day 1, 3 and 10 post-instillation. | * LDH in BALF was significantly elevated for MMA-SS exposure at all time points but only on day 1 and 3 following GMA-MS exposure where it had returned to control levels by day 10.
* Neutrophils in BAL increased significantly for both MS and SS on days 1 and 3 but only for SS on day 10.
* AMs in BAL were not significantly increased at all for MS but were significantly increased for SS exposure on day 3 and 10.
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| Stainless steel welding fumes inhaled in mice(Falcone et al. 2017) | GMAW-SS welding fumes: 10-50 nm particles linked together in chains (SEM); mass mean aerodynamic diameter = 350 nm; 57% Fe, mostly water insoluble. | Natural: Gas metal arc-stainless steel (GMAW-SS) welding fumes, produced *in situ* for inhalational exposure. | A/J Mice, whole-body inhalation. Inhaled dose: Day 1 intra-peritoneal injection of MCA (chemical initiator). Day 7 exposed to GMAW-SS welding fumes to 4 hours/day for days/week for 9 weeks at 32.3 mg/m3.Euthanized and organs excised at 30 weeks post initiation. | * Fe level in lung after 4 hours was 15.12 µg/lung (c.f. air value was 8.97 µg/lung). Cr, Mn and Ni also substantially increased in lung.
* Total mass of 10.1 µg metal deposited per lung in 4 hour exposure.
* Total mouse exposure estimated deposition of 254.4 µg. (occupationally relevant dose).
* GMAW-SS welding fume significantly promoted lung tumours in the A/J mouse after initiation with MCA. Without an induced initiation there was no effect of welding fumes alone.
* For induced mice tumour multiplicity was 7.93 for air exposure and 16.11 for GMAW-SS exposure (p<0.0001).
* Comparing the morphology of MCA/air exposed mice and MCA/GMAW-SS exposed mice there was a significant increase in lymphoid infiltrates (p<0.0002), presence of foreign material (p<0.0002), hyperplasia (p<0.004), adenoma (p<0.05) and total lesions (p<0.004).
* These findings support the association between lung cancer and welding.
* Fresh welding fumes used in an inhalation study are more reactive due to an increase in ROS compared to 'aged' welding fumes used in instillation studies.
* Formation of adenomas in mice is clinically relevant as these adenomas are often precursors to lung adenocarcinomas in humans.
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| Stainless steel welding fumes aspirated in mice (Zeidler-Erdely et al. 2011) | MMA-SS welding fumes: 41% of sample is Fe; 0.845 µg/sample soluble Fe; 343 µg/sample insoluble Fe. No additional PM property details provided. | Natural: Welding fumes filter collected then extracted for exposure. Manual metal arc – stainless steel (MMA-SS) welding. | A/J and B6 mice, pharyngeal aspiration.Aspiration dose: exposed once per month for 4 months to 20 mg/kg MMA-SS welding fumes (estimate of 4 year exposure in full-time welder). Euthanized at 78 weeks post first dose.   | * No significant change in Fe concentrations in the liver or kidney following instillation of a suspension of SS welding fume particles.
* There was a significant increase in tumour multiplicity in the A/J strain at 78 weeks.
 |
| Stainless steel welding fumes and iron oxide particles instilled in mice(Falcone et al. 2018b) | Fe2O3: SSA = 6.0 m2/g; hydrodynamic diameter ~650 nm; Zeta potential ~31.7 mV. | Natural: GMAW-SS welding fumes collected on filters and extracted.Additional metal oxides were purchased from chemical suppliers (for comparison) (Sigma Aldrich, USA). | A/J mice, oropharyngeal aspiration.Exposure levels: low dose Fe2O3 1 mg/mouse; high dose Fe2O3 2 mg/mouse; GMAW-SS 1.7 mg/mouse.Euthanized at various time points.  | * BAL fluid LDH activity was significantly increased for low dose (>2 fold), high dose Fe2O3 (~3 fold) and GMAW-SS fume (5 fold) at 7 days post instillation. Welding fume and high dose iron remaining significant at 84 days post instillation.
* Total BAL cells and macrophages were still significantly increased for high dose iron right at 84 days post instillation (low dose significantly increased to 28 days).
* Neutrophils in BAL were significantly increased for high and low dose iron at 1 day and 7 days post instillation.
* Among the metal components testing (which included nickel and chromium) only Fe2O3 significantly promoted lung tumour formation following MCA initiation compared to MCA/sham (p<0.0001).
* At doses representative of the total GMAW-SS welding fume by weight Fe2O3 was the greatest contributor to lung inflammation and the only metal oxide tested to promote lung tumours in mice.
* The pneumotoxicity for the GMAW-SS fume was greater than any of the individual metal oxide components tested.
 |
| Stainless steel welding fumes via inhalation in rats(Sriram et al. 2015) | Regular voltage (RV) welding produced fumes which were 53.5% Fe and high voltage (HV) produced fumed which were 57.0% Fe. | Natural: gas–metal arc-stainless steel(GMA-SS) welding at standard/regular voltage (25 V) or high voltage (30 V). | Sprague-Dawley rats, whole body exposure inhalation.Exposure levels: 40 mg/m3, 3h/day, 5 day/week, for 2 weeks. Exposure to freshly produced particles. | * Rats exposed to RV fumes showed a significant increase in the expression of divalent metal transporter-1 mRNA, Ccl2 mRNA, TNFα mRNA and Nos2 mRNA whereas HV fumes were not significant for any of these.
* Changing voltage altered the component of soluble Mn in the sample which may have affected toxicity of these samples.
 |
| Mild steel welding fumes inhaled in mice(Falcone et al. 2018a) | GMAW-MS welding fumes: Most particles 0.1-1 µm; mass mean aerodynamic diameter 0.31 µm; Fe by weight was 83.67%, Mn was 14.33%. | Natural: Gas metal arc welding-mild steel (GMAW-MS) fumes, produced *in situ* for inhalational exposure. | A/J mice, while body inhalation.Inhaled dose: Day 1 intra-peritoneal injection of MCA (chemical initiator). Day 7 exposed to GMA-SS welding fumes to 4 hours/day for days/week for 8 weeks at 34.5 mg/m3.Euthanized and organs excised at 30 weeks post initiation. | * Fe in mouse lung 14.62 µg/lung vs 9.45 µg/lung for air exposure. Mn 0.88 µg/lung vs 0.01 µg/lung for air.
* MS fumes significantly promoted tumours in A/J mice initiated with MCA with a lung tumour multiplicity of 21.86 ± 1.50 for MCA/GMAW-MS fumes vs 8.34 ± 0.59 for MCA/air.
* The histopathological findings showed a significant increase (all p<0.0001) over MCA/air for MCA/GMAW-MS for presence of foreign material, hyperplasia, hyperplasia severity, adenoma and total number of lesions.
* MS fumes were a tumour promoter despite a lack of chronic lung inflammation and no known carcinogenic metals in the fumes. Authors suggest Fe may actually be responsible for the effects via production of ROS.
 |
| Mild steel welding fumes in instillation in mice with subsequent infection(Suri et al. 2016) | MS fumes were 12.4% iron by weight. | Natural: welding fumes collected on filters, extracted and stored until use. | CD1 mice, intranasal instillation.Exposure levels: 600 µg in PBS single dose or divided doses; 24 hours later infected with *S pneumoniae*.Euthanized 24 post infection. | * BALF and lung pneumococcal colony forming units (CFU) values were significantly increased in mice with MS instillation before infection.
* Intranasal instillation of MS followed by infection resulted in a 50-175 fold increase in lung CFU values in mice.
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| Geogenic dust aspiration in mice(DeWitt et al. 2017) | Geogenic dust (naturally collected). 4.05 μm average diameter. 33,266 μg/g Fe (second highest metal behind Al). | Natural: geogenic dust (ground collected). Size extracted to concentrate ~ 4 μm particles.  | B6C3F1 mice, oropharyngeal aspiration. Aspirated dose: 0, 0.01, 0.1, 1.0, 10, or 100 mg/kg body weight once weekly for 4 weeks.Euthanized 1 day after final dose. | * Mean corpuscular volume decreased by ~ 20% for all concentrations.
* Alanine aminotransferase decreased between 45.6%-62.6% for all concentrations (increased levels suggest liver toxicity, decreasing cause is not clear).
* Creatinine levels increased by 10% for the 10 and 100 mg/kg dosage groups (increased levels suggest nephrotoxicity).
* Small decreases (<30%) were seen in CD4+, CD8+, CD4+/CD8+, CD4-/CD8- in the spleen across a range of concentrations with no clear concentration trend.
* Plaque forming cells/million spleen cells decreased by 39.9-62.4% for all concentrations tested.
* Natural killer cell activity and IgM, IgG antibody production was not altered.
* Conclusion: overall immune suppression.
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| Occupational exposure in humans to road pollution(Baccarelli et al. 2014) | Road pollution PM: No PM size or property details provided. | Natural: workplace exposure to PM from road pollution.  | Human truck drivers, inhalational exposure N=60 (additional 60 controls).Exposure levels: individually determined by personal exposure device.Lung function measurement. | * Fe was negatively associated with forced expiratory volume 1 (FEV1) among truck drivers (-4.24% 95%CI -7.55 to -0.8). Negative associations for FEV1 became more strongly negative when only considering non-smokers and those with a BMI below median.
* Other metals including Si, Al, Ca and Ti also showed negative associations.
 |
| Occupational exposure to iron oxide pigments(Pelclova et al. 2016) | Dust samples from the workplace identified Fe2O3 and Fe3O4 as the major components of dust with one sample (yellow dust) containing FeO(OH). | Natural: workplace exposure to iron oxide pigments (mean 10 years of exposure).  | Human workers employed in an iron oxide pigment factory. N=14 (exposed).  | * Levels of malondialdehyde, 4-hydroxy-trans-hexenale, 4-hydroxy-trans-nonenale, 8-isoProstaglandin F2alpha, 8-hydroxy-2-deoxyguanosine, 8-hydroxyguanosine, hydroxymethyl uracil, o-tyrosine, 3-chlorotyrosine and nitrotyrosine in exhaled breath condensate were all significantly (P<0.001) elevated over controls in individuals with workplace exposure to iron oxide.
* Aldehydes C6-C10 in exhaled breath condensate were significantly elevated over controls (p<0.001). Aldehyde C11 was also significantly increased (P<0.01). The increase for aldehyde C12 was not significant.
* Oxidative stress markers in urine were not statistically different in workers and controls.
 |
| Ambient urban PM, human exposure.(Wu et al. 2016b) | Ambient urban PM: No details of iron content or PM property details provided. | Natural: ambient urban environment PM. Longitudinal study examining the same group of individuals living in a rural setting then again following a move to an urban setting. | Human, ambient environment PM. Exposure levels: suburban PM10 135.4 µg/m3, PM2.5 56.1 µg/m3; urban 1: PM10 111.3 µg/m3, PM2.5 78.1 µg/m3; urban 2: PM10 129.4 µg/m3, PM2.5 59.9 µg/m3. | * PM10 was significantly positively associated with ET-1 levels at 1 day and 2 day averages.
* PM2.5 from dust/soil was significantly associated with increasing ET-1 at 1 day average. ET-1 mediates vasoconstriction and concentrations changes are considered an early marker of vascular dysfunction.
* When the influence of individual metals was modelled iron showed a positive (non-significant) correlation with % change in ET-1, % change in E-selectin, % change in ICAM-1 and % change in VCAM-1.
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| Ambient urban PM, human exposure(Rosa et al. 2014) | Ambient urban PM: Fe 93.8 ng/m3. No PM size or property details provided. | Natural: ambient urban pollution in New York, USA. | Human children (9-11 years old), ambient environment PM. N=192.Exposure levels: ambient exposure (no total PM concentrations provided). | * Iron in PM2.5 averaged over 9 days was a significant predictor of FENO50 β=0.375 (95%CI 0.053, 0.696) and JNO β=0.185 (95% CI 0.035, 0.335).
* Iron remained significant after adjusting for covariates FENO50 β=0.406 (95% CI 0.060, 0.751) and JNO β=0.679 (95% CI 1.33, 1.225).
* Of the four metals analysed only Fe was a significant predictor of JNO levels.
* Conclusion was that short term exposure to ambient transition metal fractions of PM2.5 may increase lung inflammation with different metals having varied effects on NO production in the airways.
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| Ambient urban exposure to general pollution(Boogaard et al. 2013) | Ambient urban PM: No PM size or property details provided. | Natural: ambient urban pollution in 12 locations in the Netherlands. Exposure levels determined close to participants living locations. | Human adults, ambient environmental PM N=661 (re-examined).Exposure levels: PM10 26.6 µg/m3; PM2.5 16.0 µg/m3; Fe 686.0 ng/m3.Lung function measurement. | * A regression analysis adjusted for covariates found a reduction in Fe was associated with a statistically significant improvement in forced vital capacity (soot, NO2, NOx and Cu reduction showed a similar association).
 |
| Ambient urban PM exposure in humans(Wu et al. 2015; Wu et al. 2016a) | Median Fe concentration in PM: suburban = 0.74 µg/m3; urban period 1 = 0.72 µg/m3; urban period 2 = 0.55 µg/m3. | Natural: Ambient pollution exposure. Students in China spent 2 years at suburban campus then 2 years at urban campus. | Human adults, ambient environmental, China. Students N=40.Determined daily air pollution analysis at both campuses.Blood collection and analysis. | * Fe in PM2.5 showed a significant positive correlation with oxidised low density lipoproteins (Ox-LDL) with a 1 day lag.
* Ox-LDL is a biomarker of oxidative stress which is associated with the initiation of atherosclerosis.
* Iron in PM2.5 was significantly associated with an increase in major anti-oxidant enzymes superoxide dismutase and glutathione peroxidase 1 at 3 -day moving average.
* Associations based on exposure metrics of 2-7 day moving averages were stronger than those based on single day metrics, suggesting cumulative exposure might better capture air pollution effects than single day exposures.
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| Ambient urban PM, human asthmatic exposure(Godri Pollitt et al. 2016) | Ambient urban PM: No PM size or property details provided. | Natural: ambient urban pollution in Montreal, Canada. | Human children (8-13 years old) diagnosed with asthma, ambient environment PM. N=217.Exposure levels: individually determined by personal exposure device. Fe exposure mean 68.3 ng/m3.Lung function measurement. | * Increased FeNO in the presence of Fe with a 1 day lag period.
* General results showing PM increases asthma in children.
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**Table SM5: Summary of epidemiological studies on particulate matter with specific data on iron. Abbreviations: CI – confidence interval; COPD: chronic obstructive pulmonary disease; CV – cardiovascular; EC – elemental carbon; OC – organic carbon; OR – odds ratio; PM – particulate matter; RR – risk ratio; SIR – standardized incidence ratio; SMR – standardized mortality ratio.**

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| **Study and reference** | **Particle type, size, concentration and source** | **Summary of findings for iron** |
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| California 6 cities2000-2003Children (<19 years) hospital admissions data(Ostro et al. 2009) | PM2.5 14.8 – 28.8 µg/m3.Fe 0.13 µg/m3 (full year mean).No PM source determination in this study. | * Acute exposure to PM2.5 was associated with respiratory hospital admission for <19 years and <5 years, with children <5 years being particularly sensitive.
* Significant correlations for EC, OC, NO3, SO4, Fe and Si.
* Fe had a significant correlation with respiratory hospitalisation following a 3-day lag for <5 years but not 5-18 years with no direct correlation seen for pneumonia or bronchitis.
* Only looked at lags of 0-3 days, it is possible that additional immune suppression may occur following a longer lag period.
 |
| Meta-regression analysis of ambient PM2.5 pollution related mortality.41 studies included in meta-regression.(Achilleos et al. 2017) | Ambient PM2.5.PM size, source and additional properties vary significantly across all studies included in the meta-analysis. | * Huge amount of variability between studies particularly which components caused the highest levels of mortality.
* Examining all the data there was a: 0.89% increase in all-cause mortality per 10 µg/m3; 0.80% increase in CV mortality per 10 µg/m3; 1.10% increase in respiratory mortality per 10 µg/m3.
* There was a positive association between Fe and all-cause mortality and CV mortality (but not respiratory), however, these were not significant due to large CI.
* Interestingly despite the associations with Fe (which were mentioned in the results section) this paper failed to mention Fe in the discussion at all.
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| Chile Hospital ED presentations.(Cakmak et al. 2009) | Urban PM exposure, tracked against daily pollution levels and contaminant type. | * RR of total non-accidental morbidity for Fe as a pollutant was 1.045 (95% CI 1.013-1.078) and respiratory morbidity for Fe exposure was RR = 1.084 (95% CI 1.040-1.129) both with a 1 day lag. The elderly and very young were particularly susceptible in this study
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| Steel workers, AlbaniaModified American Thoracic Society questionnaire with pulmonary function test. N=459.(Bala and Tabaku 2010) | Steel workplace exposure, classified by type of work: iron-steel production; iron-steel processing; ferrochrome production.No PM size determination in this study. | * High rates of COPD among steel-workers (27.1%) with the highest rates among smelters.
* Rates were highest among current smokers (29.8%), then ex-smokers (21.7%) and non-smokers (18.4%).
* Relative risk of developing COPD for iron-steel workers was calculated as 5.51 (CI 95% 3.98-6.73) with an age-smoking adjusted rate of 5.11 (CI 95% 3.30-6.50).
 |
| Electric arc furnace workers, Italy.Company records in association with health exemptions and morbidity records. N=331 (29 deaths).(Cappelletti et al. 2016) | Foundry workplace exposure (median exposure = 5.5 years).Fe in foundry dust average of 197,000 mg/kg.No PM size determination in this study. | * There was a significant increase for malignant tumours of the larynx, trachea, bronchi, and lungs (SMR=3.35, p=0.010). Interestingly there were higher rates for those exposed for 1-5 years (SMR=4.35) and 5-10 years (SMR=4.11) than for >10 years (SMR=2.06).
* Morbidity analysis (using exemption of health fees which may under estimate actual cases) determined a significant increase (p>0.02) in relative risk for diabetes (RR=2.24) rheumatoid arthritis (RR=6.18), non-complicated hypertension (RR=2.23) and complicated hypertension (RR=2.01). When adjusting for age cardiovascular diseases also became significant (RR=1.74).
 |
| Taconite mining workers, Minnesota, USA.N=40,720.(Allen et al. 2015) | Mining workplace exposure (median exposure = 5.5 years).Taconite is a low grade iron ore with ~30% iron.No PM size determination in this study. | * 5,700 cancers identified of which 973 were lung cancer and 51 were mesotheliomas.
* Mesothelioma SIR = 2.4 (95% CI 1.8-3.2); lung SIR = 1.3 (95% CI 1.2-1.4); laryngeal SIR = 1.4 (95% CI 1.1-1.7); stomach SIR = 1.4 (95% CI 1.1-1.6); bladder SIR 1.1 (95% CI 1.0-1.2).
 |
| Europe + UK Case control study, welding and lung cancer. Face-to-face interviews. 1998-2001. N=2197 (incidents of lung cancer <75 years age), 2295 controls.Attempt to determine influence of asbestos and smoking on welding fumes and lung cancer.(Mannetje et al. 2012) | Exposure based on types of fumes exposed to e.g. welding fumes from gas welding or arc welding, chromium fume exposure.No PM size or concentration determination in this study. | * After adjustment for age, centre, education and smoking an OR of 1.19 was obtained for individuals ever exposed to welding fumes and 1.37 for those whose occupation was a welder/flame cutter.
* Adjustment for asbestos and silica exposure had little effect on the risk estimates suggesting minimal confounding from these factors.
* Adjustment for chromium reduced the risk element by ~ 40%, however, adjustment for nickel, cadmium and ionizing radiation exposure did not appreciably change the risk.
* There was a clear relationship between years of exposure to welding and risk, with the highest risk in those with more than 25 years of exposure to welding fumes (OR = 1.38).
* Lifetime exposed hours also showed a gradual increasing relationship with more than 7000 hours welding OR = 1.31.
* Welders with Cr exposure had a higher risk (OR 1.34 95% CI: 1.04, 1.71) than welders without Cr exposure (OR 1.14 95% CI: 0.95, 1.36).
* Welders without Cr exposure and > 25 years of exposure had an increased OR of 1.48 (95% CI: 1.11, 1.97).
* Smoking did not affect the risk estimate for welding fumes and did not appear to be an effect modifier in the association between welding fumes and lung cancer.
* Conclusion: 4% of lung cancers among the men in this population study was caused by exposure to welding fumes. There was no confounding by asbestos or smoking.
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